Docket No.: REGIM 3.3-025

Application No.: 10/501,065

IN THE CLAIMS

- 1. (currently amended) A method for producing nonhuman nonprimate mammalian embryos comprising the following steps:
 - (a) determining asynchrony of development (T) between two embryos of the same species and of the same age:
 - (i) wherein a first embryo is produced by crossing at time t₀ a vasectomized male with a female, said first embryo being cultured, or manipulated in vitro, or cultured and manipulated in vitro; and
 - (ii) a second embryo being produced by crossing at time t₀ a fertile male with a female, said second embryo being normally fertilized and obtained by parthenogenetic activation,

the determination taking place at on or before the day of uterine implantation of said second embryo;

(b) transferring an embryo which is cultured, or manipulated, or cultured and manipulated in vitro into a uterus of a recipient female who was crossed with a vasectomized male at time $t = t_0 + T + \frac{(+/-25\% T)}{(+/-25\% T)}$ wherein T is at least 15 hours; and wherein said males, said females, and said embryos are all of the same species.

(+/-25% T).

- 2. (previously presented) The method of claim 1, wherein said first embryo is cultured, or manipulated, or cultured and manipulated in vitro up to the day of implantation.
- 3. (previously presented) The method of claim 1, wherein said determination is carried out at a stage of development chosen from a 1 cell stage, 2 cell stage, 4 cell stage, 8 cell stage, 16 cell stage, morula stage and blastocyst stage.
- 4. (previously presented) The method of claim 3, wherein said determination is carried out at the blastocyst stage.

Docket No.: REGIM 3.3-025

Application No.: 10/501,065

5. (previously presented) The method of claim 1, wherein said determination of the asynchrony of development T is carried out by cell counting.

- 6. cancelled
- 7. (previously presented) The method of claim 1, wherein said asynchrony of development T is about 24 hours.
- 8. (previously presented) The method of claim 1, wherein said embryo transferred in step b) is cultured under the same conditions as said first embryo.
- 9. (previously presented) The method of claim 1, wherein said embryo transferred in step b) is at a 1 cell stage.
- 10. (previously presented) The method of claim 1, wherein said embryo transferred in step b) is at a 2 cell stage.
- 11. (previously presented) The method of claim 1, wherein said embryo transferred in step b) is at a 4 cell stage.
- 12. (previously presented) The method of claim 1, wherein said transferred embryo develops into a fetus.
- 13. (previously presented) The method of claim 12, wherein said fetus develops into a newborn.
- 14. (previously presented) The method of claim 1, wherein said embryo cultured, or manipulated, or cultured and manipulated in vitro is a transgenic embryo.
- 15. (previously presented) The method of claim 1, wherein said embryo cultured, or manipulated, or cultured and manipulated in vitro is a reconstituted embryo obtained by nuclear transfer.
- 16. (previously presented) The method of claim 1, wherein said embryo cultured, or manipulated, or cultured and manipulated in vitro is a reconstituted transgenic embryo obtained by nuclear transfer.
- 17. (currently amended) The method of claim 1, wherein said mammal is selected from the group consisting of rodents,

lagomorphs, hoofed animals, <u>and</u> equine animals. and non-human primates.

- 18. (previously presented) The method of claim 17, wherein said mammal is a rodent selected from the group consisting of mice, rats, hamsters, and guinea pigs.
- 19. (previously presented) The method of claim 17, wherein said hoofed animal is selected from the group consisting of bovines, ovines, caprines and porcines.
- 20. (previously presented) The method of claim 17, wherein said lagomorph is rabbit.
 - 21. 24. cancelled
- 25. (currently amended) An *in vitro* method for cloning a nonhuman mammal by nuclear transfer wherein the method comprises a step of using a nonhuman nonprimate mammalian embryo according to the method of claim 1.
- 26. (currently amended) A method for producing rabbit embryos comprising the following steps:
 - (a) determining asynchrony of development (T) between two same age rabbit embryos
 - wherein a first embryo is produced by crossing at time t₀ a vasectomized male with a female, said first embryo being cultured, or manipulated, or cultured and manipulated in vitro; and
 - a second embryo is produced by crossing at time t₀ a fertile male with a female, the second embryo being normally fertilized and obtained by parthenogenetic activation;
 - said determination taking place on or before the day of uterine implantation of said second embryo normally fertilized or obtained by parthenogenetic activation;
 - (b) transferring a rabbit embryo which is cultured, or manipulated, or cultured and manipulated in vitro, no older than blastocyst stage into a uterus of a recipient female

who was crossed with a vasectomized male at time $t = t_0 + T$ $(+/- 25% T)_{+}$.

- 27. (previously presented) The method of claim 26, wherein said determination is carried out at a stage of development between days D1 and D7 post coitum.
- 28. (previously presented) The method of claim 27, wherein said determination is carried out on day D5 post coitum.
- 29. (previously presented) The method of claim 26 wherein said asynchrony of development T is about 17.25 to 28.75 hours.
- 30. (previously presented) The method of claim 26, wherein said embryo cultured, or manipulated, or cultured and manipulated in vitro is a transgenic embryo.
- 31. (previously presented) The method of claim 26 wherein said embryo cultured, or manipulated, or cultured and manipulated in vitro is a reconstituted embryo obtained by nuclear transfer.
- 32. (previously presented) The method of claim 26 wherein said embryo cultured, or manipulated, or cultured and manipulated in vitro is a reconstituted transgenic embryo obtained by nuclear transfer.
- 33. (previously presented) The method of claim 26 wherein said embryo transferred in step b) is at a 1 cell stage.
 - 34. 37. cancelled
- 38. (previously presented) An in vitro method of cloning rabbits by nuclear transfer comprising the method of claim 26.
- 39. (previously presented) An in vitro method for cloning rabbits by nuclear transfer, said method comprising the steps of:
 - a) inserting a rabbit donor cell or a rabbit donor cell nucleus into a rabbit enucleated oocyte under conditions which make it possible to obtain a reconstituted embryo;
 - b) activating the reconstituted embryo obtained in step a);

- c) transferring said reconstituted embryo into a surrogate rabbit, such that the reconstituted embryo develops into a fetus or newborn;
- whereby the method comprises or includes a method as claimed in claim 26.
- 40. (previously presented) The method of claim 39, wherein the transfer of nucleus into a recipient cytoplasm is carried out by fusion of the donor cell and of the recipient cytoplasm.
- 41. (previously presented) The method of claim 39, wherein the transfer of nucleus into a recipient cytoplasm is carried out by microinjection of the donor nucleus into the recipient cytoplasm.
- 42. (previously presented) The method of claim 39, wherein said activating the reconstituted embryo is carried out by adding simultaneously, successively or spaced out over time, to culture medium for said reconstituted embryo, at least one protein kinase inhibitor and at least one inhibitor of protein synthesis.
- 43. (currently amended) An in vitro method for cloning nonhuman-nonprimate mammals, comprising the steps of:
 - a) inserting a donor cell or a donor cell nucleus into an enucleated oocyte of a mammal of a same species or of a species different from that of the donor cell under conditions which make it possible to obtain a reconstituted embryo;
 - b) activating the reconstituted embryo obtained in step a);
 - c) transferring said reconstituted embryo into a surrogate female mammal, such that the reconstituted embryo develops into a fetus, whereby said activation is carried out by adding simultaneously, successively or spaced out over time, to culture medium for said reconstituted embryo,

Docket No.: REGIM 3.3-025

Application No.: 10/501,065

- at least one protein kinase inhibitor and at least one inhibitor of protein synthesis.
- 44. (currently amended) The method of claim 43, wherein said mammal is selected from the group consisting of rabbits, rodents, in particular rats, mice, and from bovines, ovines, caprines, porcines, and equines. and nonhuman primates.
- 45. (previously presented) The method of claim 42 wherein said protein kinase inhibitor is 6-DMAP and said inhibitor of protein synthesis is cycloheximide (CHX).
 - 46. 50. cancelled
- 51. (previously presented) The method of claim 1, wherein said embryo transferred in step b) is implanted and allowed to develop in the uterus of said recipient female.
- 52. (previously presented) The method of claim 1, wherein said female has received hormone treatment to increase ovulation.
- 53. (previously presented) The method of claim 26, wherein said embryo transferred in step b) is implanted and allowed to develop in the uterus of said recipient female.
- 54. (previously presented) The method of claim 26, wherein said female has received hormone treatment in order to increase ovulation.
- 55. (previously presented) The method of claim 26, wherein said asynchrony of development T is about 23 hours.
- 56. (previously presented) The method of claim 43, wherein said protein kinase inhibitor is 6-DMAP and said inhibitor of protein synthesis is cycloheximide (CHX).